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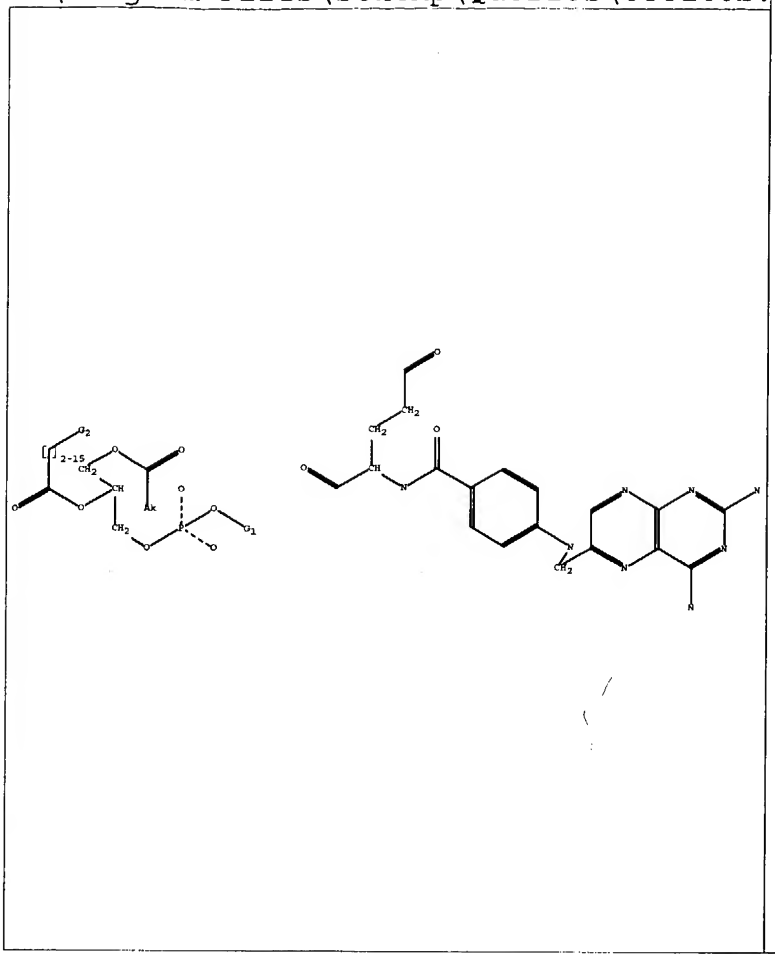

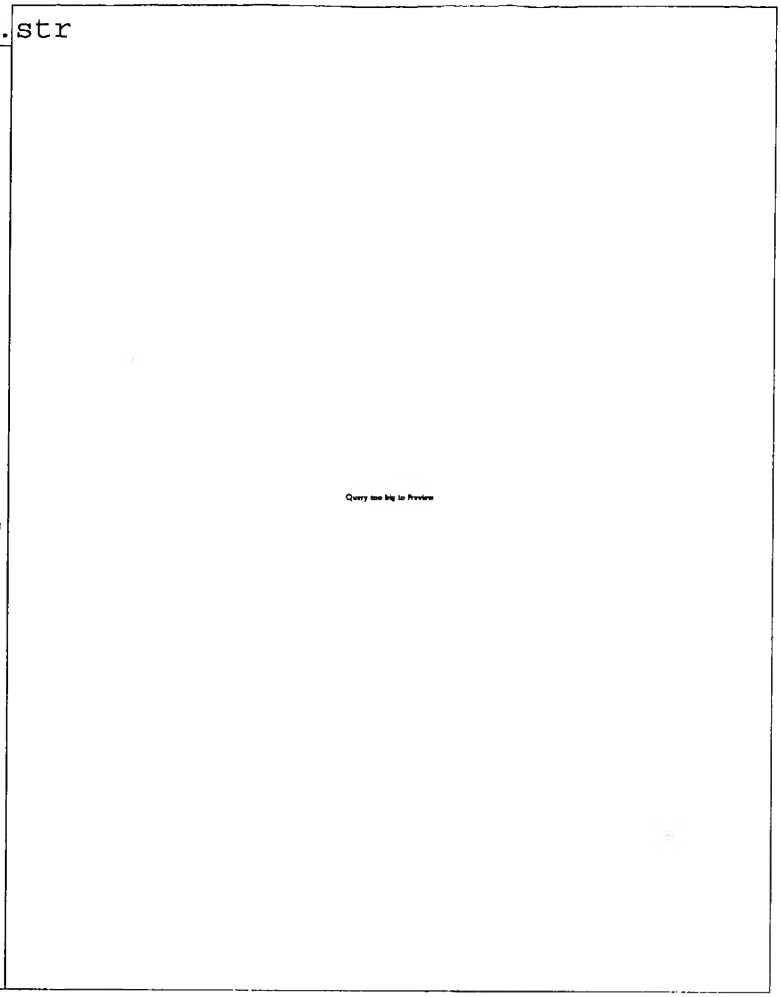


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chain bonds :
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13-14 15-16 16-17 16-18 24-40 30-33 32-42 34-41 37-43 40-41
43-44 43-45 45-46 46-47 46-48 47-51 48-52 49-50 49-52
ring bonds :
23-24 23-28 24-25 25-26 26-27 27-28 27-29 28-32 29-30 30-31
31-32 34-39 34-35 35-36 36-37 37-38 38-39
exact/norm bonds :
2-10 4-5 5-6 5-7 5-8 8-9 10-11 11-12 13-14 15-16 16-17 16-18
30-33 32-42 34-41 43-44 43-45 45-46 47-51 49-50
exact bonds :
1-2 1-15 2-3 3-4 11-13 24-40 37-43 40-41 46-47 46-48 48-52
49-52
normalized bonds :
23-24 23-28 24-25 25-26 26-27 27-28 27-29 28-32 29-30 30-31
31-32 34-39 34-35 35-36 36-37 37-38 38-39

G1:C,H

G2:C,O,S,N

Match level :

1:CLASS 2:CLASS 3:CLASS 4:CLASS 5:CLASS 6:CLASS 7:CLASS 8:CLASS
9:CLASS 10:CLASS 11:CLASS 12:CLASS 13:CLASS 14:CLASS 15:CLASS
16:CLASS 17:CLASS 18:CLASS 23:Atom 24:Atom 25:Atom 26:Atom 27:Atom
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43:CLASS 44:CLASS 45:CLASS 46:CLASS 47:CLASS 48:CLASS 49:CLASS
50:CLASS 51:CLASS 52:CLASS

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***

Structure attributes must be viewed using STN Express query preparation.

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100.0% PROCESSED 20 ITERATIONS 16 ANSWERS
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COST IN U.S. DOLLARS

| | SINCE FILE ENTRY | TOTAL SESSION |
|---|---|---|
| FULL ESTIMATED COST | 159.20 | 316.53 |

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L8 12 L7

=> d l8 1-12 ibib abs hitstr

L8 ANSWER 1 OF 12 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2001:208065 CAPLUS
DOCUMENT NUMBER: 134:242656
TITLE: Phospholipid prodrugs of anti-proliferative drugs
INVENTOR(S): Kozak, Alexander; Shapiro, Israel; Vinnikova, Marina; Ershov, Leonid; Senderikhin, Alexander; Ayalon, Oran
PATENT ASSIGNEE(S): D-Pharm Limited, Israel
SOURCE: PCT Int. Appl., 51 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| WO 2001019320 | A2 | 20010322 | WO 2000-IL562 | 20000913 |
| WO 2001019320 | A3 | 20010927 | | |
| W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM | | | | |
| RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG | | | | |
| AU 2000073093 | A5 | 20010417 | AU 2000-73093 | 20000913 |
| EP 1218013 | A2 | 20020703 | EP 2000-960946 | 20000913 |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL | | | | |
| JP 2003514770 | T2 | 20030422 | JP 2001-522958 | 20000913 |
| NZ 517522 | A | 20030829 | NZ 2000-517522 | 20000913 |
| ZA 2002001081 | A | 20030207 | ZA 2002-1081 | 20020207 |

PRIORITY APPLN. INFO.: IL 1999-131887 A 19990914
WO 2000-IL562 W 20000913

OTHER SOURCE(S): MARPAT 134:242656

AB The invention discloses prodrugs comprising anti-proliferative drugs covalently linked, via a bridging group, to a phospholipid moiety such that the active species is preferentially released, preferably by enzymic cleavage, at the required site of action. The invention further discloses pharmaceutical compns. comprising said prodrugs and the uses thereof for the treatment of diseases and disorders related to inflammatory, to degenerative or atrophic conditions, and to uncontrolled cell growth. A methotrexate derivative 1-stearoyl-2-[3-(α-dodecylate-γ-methotrexate-amido)-propanoyl]-sn-glycero-3-phosphatidylcholine was prepared, and examined for its inhibitory effect on human leukemia cell growth.

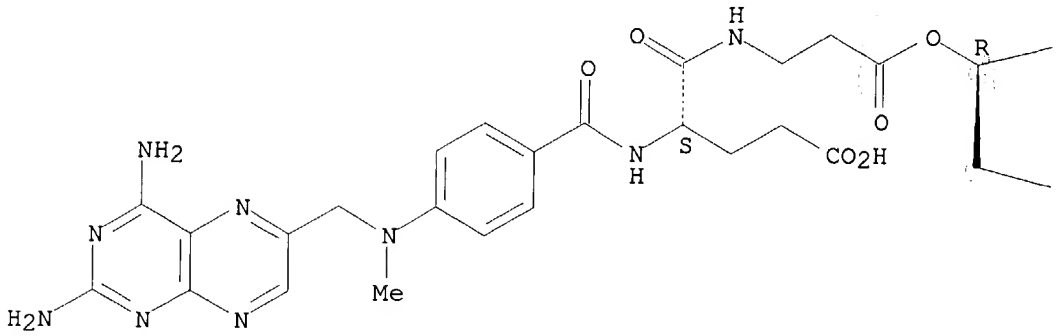
IT **330658-48-5P 330658-49-6P 330658-50-9P**
**330658-51-0P 330658-52-1P**
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); SPN (Synthetic preparation); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(preparation of phospholipid prodrugs of anti-proliferative drugs)

RN 330658-48-5 CAPLUS

CN β-Alanine, N-[4-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-L-α-glutamyl-, 2-[(1R)-1-[[[hydroxy[2-(trimethylammonio)ethoxy]phosphinyl]oxy]methyl]-2-[(1-oxooctadecyl)oxy]ethyl] ester, inner salt (9CI) (CA INDEX NAME)

Absolute stereochemistry.

PAGE 1-A



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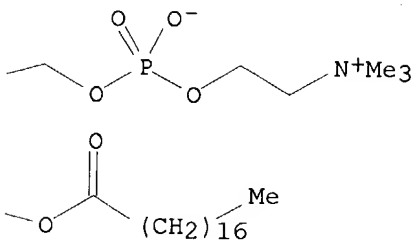
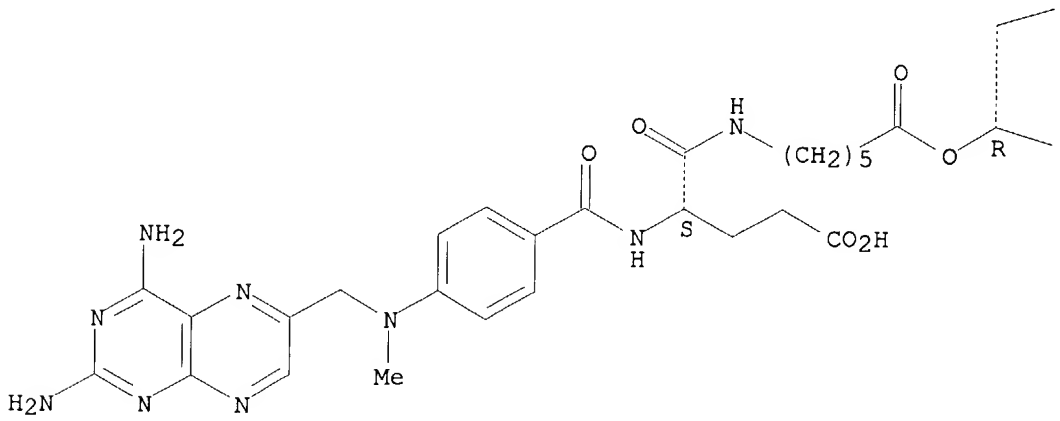


RN 330658-49-6 CAPLUS

CN 3,5,9-Trioxa-4-phosphaheptacosan-1-aminium, 7-[[6-[[(2S)-4-carboxy-2-[[4-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]amino]-1-oxobutyl]amino]-1-oxohexyl]oxy]-4-hydroxy-N,N,N-trimethyl-10-oxo-, inner salt, 4-oxide, (7R)- (9CI) (CA INDEX NAME)

Absolute stereochemistry.

PAGE 1-A



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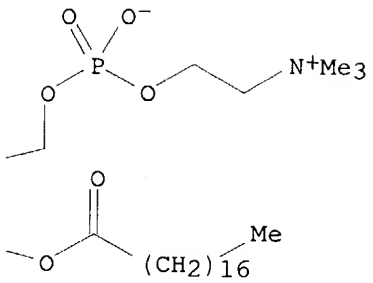
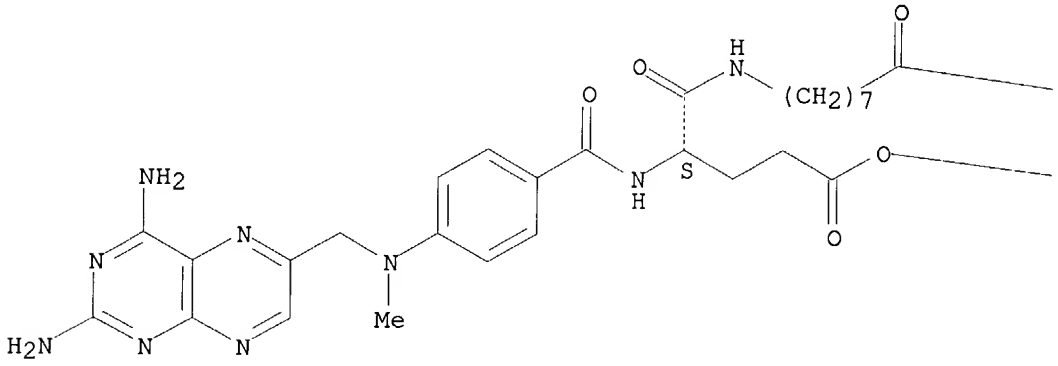


RN 330658-54-3 CAPLUS
CN 3,5,9-Trioxa-4-phosphaheptacosan-1-aminium, 7-[[8-[[(2S)-2-[[4-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]amino]-5-(dodecyloxy)-1,5-dioxopentyl]amino]-1-oxooctyl]oxy]-4-hydroxy-N,N,N-trimethyl-10-oxo-, inner salt, 4-oxide, (7R)- (9CI) (CA INDEX NAME)

Absolute stereochemistry.

PAGE 1-A



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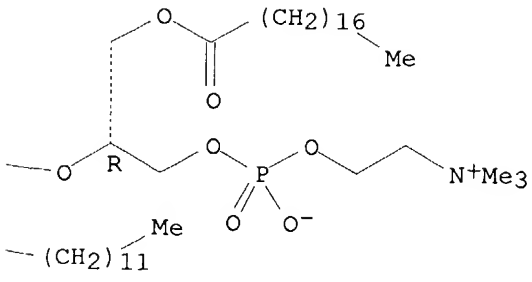


L8 ANSWER 2 OF 12 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1999:621014 CAPLUS
DOCUMENT NUMBER: 131:355990

TITLE: Interleukin-1β (IL-1β) inhibition: a possible mechanism for the anti-inflammatory potency of liposomally conjugated methotrexate formulations in arthritis

AUTHOR(S): Williams, A. S.; Jones, S. G.; Goodfellow, R. M.; Amos, N.; Williams, B. D.

CORPORATE SOURCE: Rheumatology Research Laboratory, University of Wales College of Medicine, Cardiff, CF4 4XN, UK

SOURCE: British Journal of Pharmacology (1999), 128(1), 234-240
CODEN: BJPCBM; ISSN: 0007-1188

PUBLISHER: Stockton Press

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Liposomes with conventional and long-circulation times were employed as carriers for the methotrexate derivative MTX-γ-DMPE (MTX-EPC and MTX-PEG resp.), their mechanism of action was investigated in vitro and in vivo and their therapeutic efficacy assessed using the rat collagen-induced arthritis (CIA) model. At non-toxic dose, both MTX-EPC and MTX-PEG inhibited the lipopolysaccharide (LPS) induced release of IL-1β from activated rat peritoneal macrophages (rPMΦ) in a dose and time dependent manner. Free methotrexate (MTX) was not active in this respect. After a single i.v. injection and at equivalent doses, both free MTX (500 μg) and MTX-EPC inhibited the LPS induced rise in plasma IL-1β levels observed in MTX-PEG and saline treated rats. When used to treat established CIA, MTX-EPC resulted in significantly lower clin. score (CS) (1.0±0.42 ($P<0.001$)) and hind paw diameter (HPD) (6.5±0.34 mm ($P<0.001$)) measurements than controls (3.0±0.26; 7.33±0.41 mm), after only two i.v. doses, and remained significantly lower for the entire exptl. period. By day 24 both CS (2±0.61 ($P<0.001$)) and HPD (6.97±0.25 mm ($P<0.002$)) measurements had also become significantly lower in MTX-PEG treated rats than in saline treated controls (3.62±0.17, 7.92±0.38 mm) and remained lower until day 30. Joint inflammation in MTX treated rats was completely ameliorated by day 20 but the health and well being of the animals was compromised and the experiment terminated at this time-point. Our results clearly demonstrate that both MTX-EPC and MTX-PEG liposomes have potential for development into therapeutic modalities for the treatment of inflammatory joint disease in man.

IT **97866-97-2**

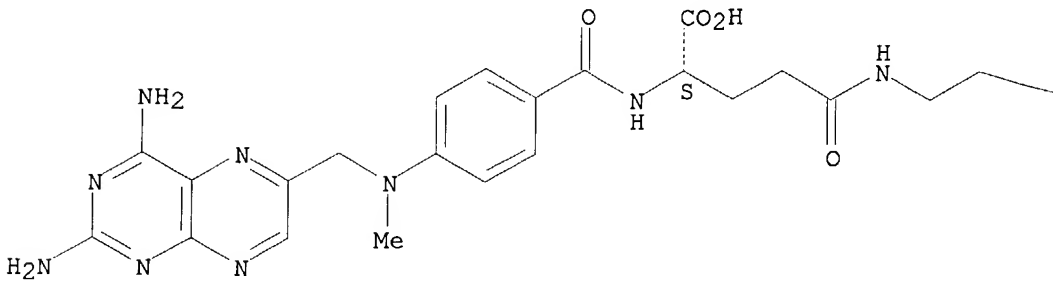
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); PEP (Physical, engineering or chemical process); THU (Therapeutic use); BIOL (Biological study); PROC (Process); USES (Uses)
(interleukin-1β inhibition as possible mechanism for the anti-inflammatory potency of liposomally conjugated methotrexate formulations in arthritis)

RN 97866-97-2 CAPLUS

CN L-Glutamine, N2-[4-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-N-[4-hydroxy-4-oxido-10-oxo-7-[(1-oxotetradecyl)oxy]-3,5,9-trioxa-4-phosphatricos-1-yl]- (9CI) (CA INDEX NAME)

Absolute stereochemistry.

PAGE 1-A



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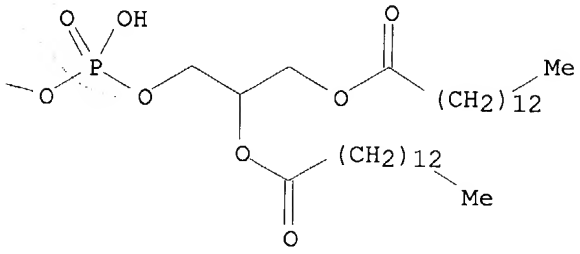


REFERENCE COUNT: 41 THERE ARE 41 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 3 OF 12 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1996:651695 CAPLUS
DOCUMENT NUMBER: 125:316588
TITLE: A single intra-articular injection of liposomally conjugated methotrexate suppresses joint inflammation in rat antigen-induced arthritis
AUTHOR(S): Williams, A. S.; Camilleri, J. P.; Goodfellow, R. M.; Williams, B. D.
CORPORATE SOURCE: College Medicine, University Wales, Health Park/Cardiff, CF4 4XN, UK
SOURCE: British Journal of Rheumatology (1996), 35(8), 719-724
CODEN: BJRHDF; ISSN: 0263-7103
PUBLISHER: Oxford University Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB In this study, the authors sought to determine whether liposomal prepns. containing a phospholipid conjugate of methotrexate and dimyristoylphosphatidylethanolamine (MTX-γ-DMPE) incorporated within their lipid membranes are effective in suppressing established joint inflammation in a monoarticular model of arthritis in the rat. Arthritis was induced in the right knee joint of Lewis rats. The rats were treated with a single intra-articular injection of either free methotrexate (MTX), liposomal MTX [MTX-multilamellar vesicles (MLV)-1.2 μm or MTX-small unilamellar vesicles (SUV)-100 nm], control liposomes (E-LIPO) or saline into the inflamed knee 7 days after arthritis induction. There was no significant difference in knee swelling in MTX-, saline- and E-LIPO-treated rats ≤21 days after treatment. However, MTX-MLV treatment produced a significant reduction in knee swelling (26.5%) 1 day after intra-articular injection compared with MTX (3.5%) and MTX-SUV (14.4%), resp. Over the next 20 days, knee swelling in MTX-MLV-treated rats fell progressively and

almost returned to normal. MTX-MLV treatment also inhibited the cellular infiltration associated with the arthritis. Large multilamellar liposomal prepns. of MTX-γ-DMPE are more effective than free MTX and MTX-SUV in suppressing inflammation. Their differential effects in treating the antigen-induced arthritis model are related to their retention within the joint space.

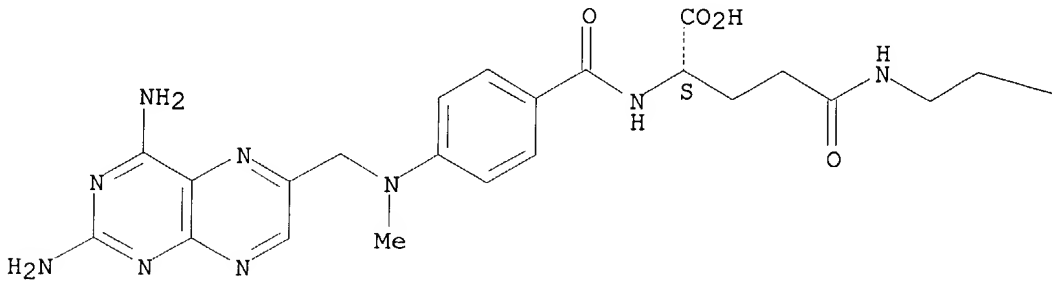
IT **97866-97-2**
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(single intra-articular injection of liposomally conjugated methotrexate suppresses joint inflammation in rat antigen-induced arthritis)

RN 97866-97-2 CAPLUS

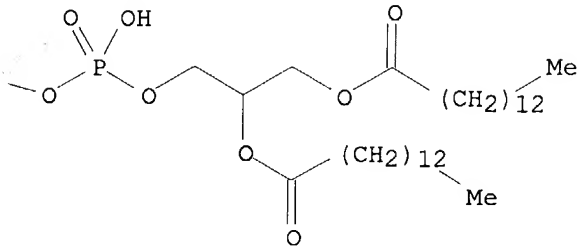
CN L-Glutamine, N2-[4-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-N-[4-hydroxy-4-oxido-10-oxo-7-[(1-oxotetradecyl)oxy]-3,5,9-trioxa-4-phosphatricos-1-yl]- (9CI) (CA INDEX NAME)

Absolute stereochemistry.

PAGE 1-A



PAGE 1-B



L8 ANSWER 4 OF 12 CAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 1994:315367 CAPLUS
DOCUMENT NUMBER: 120:315367
TITLE: Effect of three lipophilic methotrexate derivatives upon mediator release by lipopolysaccharide-stimulated rat peritoneal macrophages
AUTHOR(S): Williams, A. S.; Topley, N.; Amos, N.; Williams, B. D.
CORPORATE SOURCE: Rheumatol. Res. Lab., Univ. Wales Coll. Med., Cardiff, CF4 4XN, UK
SOURCE: Journal of Pharmacy and Pharmacology (1994), 46(4), 291-5
CODEN: JPPMAB; ISSN: 0022-3573
DOCUMENT TYPE: Journal

LANGUAGE: English

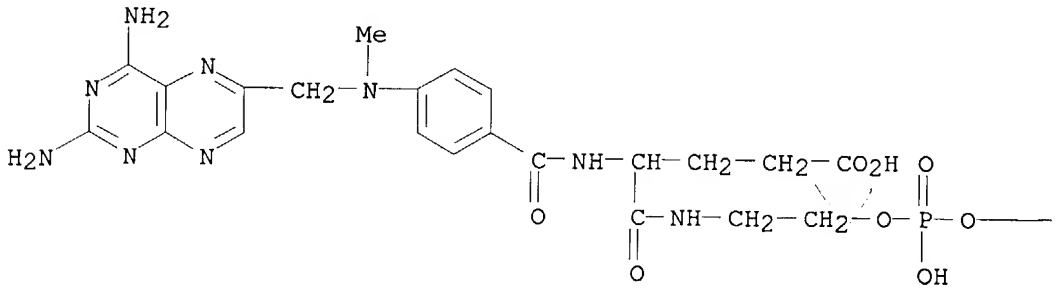
AB The ability of methotrexate and three lipophilic derivs. [methotrexate-γ-dimyristoylphosphatidylethanolamine (MγD), methotrexate-α-dimyristoylphosphatidylethanolamine (MαD) and methotrexate-α-γ-dimyristoylphosphatidylethanolamine (MαγD)] to modulate mediator release by lipopolysaccharide-stimulated rat peritoneal macrophages was investigated. At nontoxic concns., approx. 10 nmol/105 cells, MαD and MγD produced 11.06 ± 1.0 and 75.6 ± 5.2%, resp., inhibition of tumor necrosis factor (TNF) release (mean ± s.e.m., n = 4). At this same dose MαγD resulted in 68.8 ± 2.1% inhibition of TNF but cellular ATP levels were reduced by 80%. The inhibitory activity of all three derivs. was dose-dependent. Non-derivatized methotrexate at a concentration of 25 nmol/105 cells had no inhibitory effect upon TNF release (14.7 ± 0.8%, n = 3). Determination of prostaglandin E2 (PGE2) levels in the same samples demonstrated that all three conjugates were powerful inhibitors of prostaglandin release. At a quarter of the conjugate concns. described above the monoamides MαD (3.1 nmol/105 cells) and MγD (2.5 nmol/105 cells) maintained their effects on PGE2 production with 73 ± 2.3 and 71 ± 2.0% (n = 4) inhibition, resp. At this lower concentration, however, the diamide MαγD (3.1 nmol/105 cells) was less effective in reducing the amount of PGE2 released from the macrophages (29 ± 18%, n = 4). Maximal PGE2 inhibition by each of the conjugates was attained at approx. 5 nmol/105 cells. Unconjugated methotrexate (range of 2.5-20 nmol/105 cells) did not inhibit the release of PGE2 from lipopolysaccharide-stimulated macrophages.

IT **97850-22-1 97866-97-2 97866-98-3**
RL: BIOL (Biological study)
(prostaglandin E2 release from peritoneal macrophages response to)

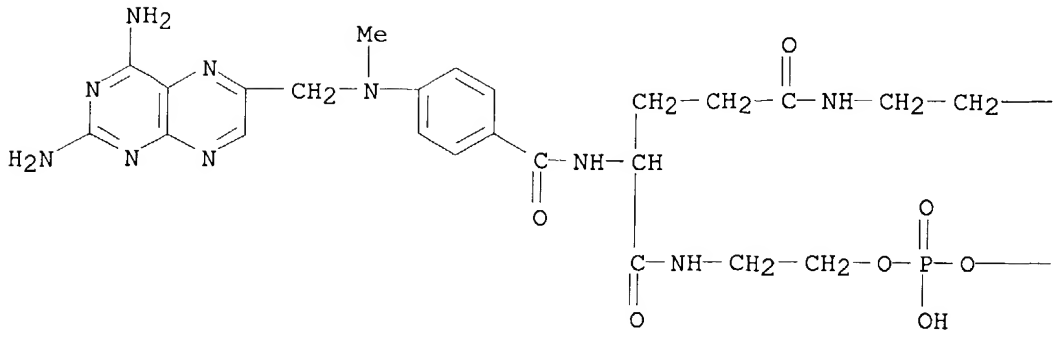
RN 97850-22-1 CAPLUS

CN 9,11,15-Trioxa-6-aza-10-phosphanonacosanoic acid, 4-[[4-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]amino]-10-hydroxy-5,16-dioxo-13-[(1-oxotetradecyl)oxy]-, 10-oxide (9CI) (CA INDEX NAME)

PAGE 1-A



PAGE 1-A



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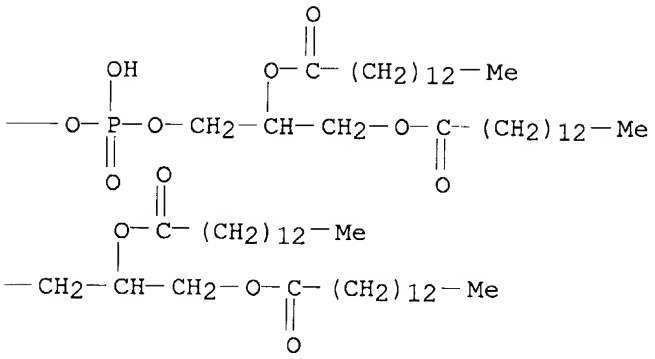


L8 ANSWER 5 OF 12 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1994:200274 CAPLUS
DOCUMENT NUMBER: 120:200274
TITLE: Effect of liposomally encapsulated MTX-DMPE conjugates upon TNFα and PGE2 release by lipopolysaccharide stimulated rat peritoneal macrophages
AUTHOR(S): Williams, Anwen S.; Topley, N.; Williams, B. D.
CORPORATE SOURCE: Rheumatology Research Laboratory, University of Wales College of Medicine, Heath Park, Cardiff, CF4 4XN, UK
SOURCE: Biochimica et Biophysica Acta (1994), 1225(2), 217-22
CODEN: BBACAQ; ISSN: 0006-3002
DOCUMENT TYPE: Journal
LANGUAGE: English

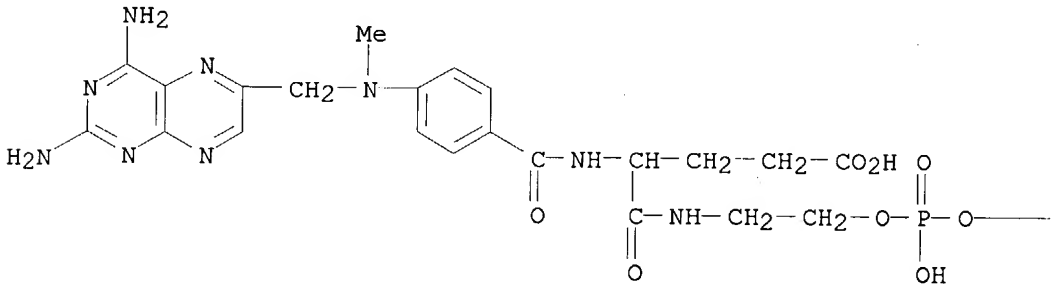
AB The ability of liposomally encapsulated prepns. of methotrexate (MTX) and three of its lipophilic derivs. (MTX-γ-DMPE, MTX-α-DMPE and MTX-α,γ-diDMPE) (DMPE = dimyristoylphophatidylethanolamine) to alter mediator release by lipopolysaccharide (LPS)-stimulated rat peritoneal macrophages (PMΘ) was investigated. The viability of these macrophages when incubated with approx. 6.0 nmol/105 cells of the resp. liposomal prepns. (MTX-LIPO, MTX-γ-LIPO, MTX-α-LIPO and MTX-di-LIPO) for 20 h was greater than 80%. Treatment of macrophages, which had been incubated with MTX-α-LIPO (5.5 nmol/105 cells), MTX-γ-LIPO (6.9 nmol/105 cells) and MTX-di-LIPO (4.5 nmol/105 cells) for 20 h, with antibody-coated sheep red blood cells resulted in

105±9.6%, 80.6±5.6% and 91±11.4% phagocytosis resp. (mean±S.E.M.). At similar concns. of MTX-α-LIPO, MTX-γ-LIPO and MTX-di-LIPO (6.5 nmol/105 cells), PGE2 release from LPS-stimulated rat peritoneal macrophages was inhibited by 85.3±3.7%, 68.7±0.6% and 88.8±2.2%, resp. (mean±S.E.M., n = 4). Incubation of these macrophages with 12, 10 and 9.4 nmol/105 cells of the resp. liposomal prepns. resulted in 89±3.3%, 62±5.5% and 85±3.9% inhibition of TNFα release (mean±S.E.M., n = 4). However, at this concentration MTX-di-LIPO was toxic. Neither MTX (20-2.5 nmol/105 cells) nor MTX-LIPO (5.6 nmol/105 cells) affected TNFα release from LPS-stimulated macrophages. While free MTX was also ineffective at inhibiting PGE2 from these cells, incubation with MTX-LIPO at the above concentration resulted in 76.9±2.6% inhibition of the prostaglandins release.

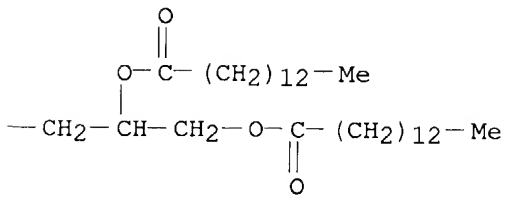
IT **97850-22-1 97866-97-2 97866-98-3**
RL: BIOL (Biological study)
(liposome-encapsulated, PGE2 and TNFα release by lipopolysaccharide stimulated peritoneal macrophages response to)

RN 97850-22-1 CAPLUS
CN 9,11,15-Trioxa-6-aza-10-phosphanonacosanoic acid, 4-[[4-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]amino]-10-hydroxy-5,16-dioxo-13-[(1-oxotetradecyl)oxy]-, 10-oxide (9CI) (CA INDEX NAME)

PAGE 1-A

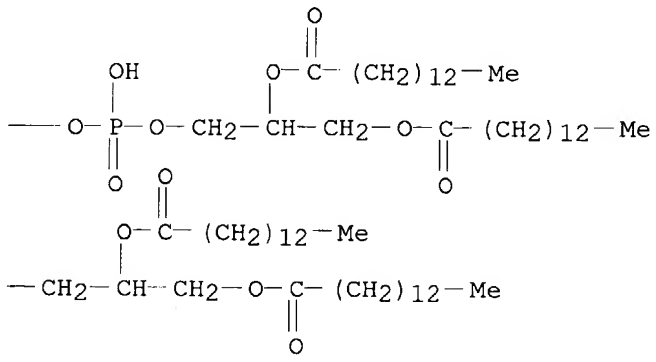


PAGE 1-B



RN 97866-97-2 CAPLUS
CN L-Glutamine, N2-[4-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-N-[4-hydroxy-4-oxido-10-oxo-7-[(1-oxotetradecyl)oxy]-3,5,9-trioxa-4-phosphatricos-1-yl]- (9CI) (CA INDEX NAME)

Absolute stereochemistry.



L8 ANSWER 6 OF 12 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1993:11606 CAPLUS
DOCUMENT NUMBER: 118:11606
TITLE: Synthesis of methotrexate-dimyristoylphosphatidylethanolamine analogs and characterization of methotrexate release in vitro
AUTHOR(S): Williams, Anwen S.; Love, W. G.; Williams, B. D.
CORPORATE SOURCE: Coll. Med., Univ. Wales, Cardiff, CF4 4XN, UK
SOURCE: International Journal of Pharmaceutics (1992), 85(1-3), 189-97
CODEN: IJPHDE; ISSN: 0378-5173
DOCUMENT TYPE: Journal
LANGUAGE: English

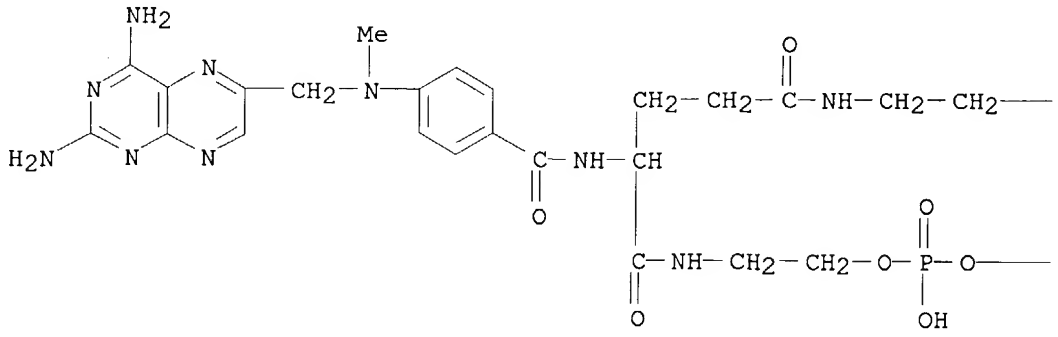
AB Lipophilic amide derivs. of methotrexate (MTX) were synthesized by covalent linkage to dimyristoylphosphatidylethanolamine (DMPE). These derivs. were characterized by IR spectroscopy, TLC and colorimetrically as MTX-γ-DMPE (B), MTX-α-DMPE (C) and MTX-α,γ-diDMPE (D). The in vitro release of free drug from each of the conjugates was determined by HPLC after incubation in phosphate buffer pH 7.4 at 37°. MTX-diDMPE (D) was most stable while the mono-substituted derivs. (B and C) released free drug more readily (t10% = 10, 2.1 and 1.3 days, resp.). The susceptibility of MTX-gamma-DMPE to hydrolysis under more physiol. conditions was also investigated. In fresh human plasma and in the presence of high esterase concns. (10 U/mL), the rate of hydrolysis was increased (t10% 19 and 1.7 h). Furthermore, MTX was released from its MTX-γ-DMPE derivative more rapidly at alkaline pH values than under acidic conditions (pH 8.7, t10% = 1.4 days and pH 2.3, t10% = 11 days).

IT **97850-22-1P 97866-97-2P 97866-98-3P**
RL: SPN (Synthetic preparation); PREP (Preparation)
(preparation and drug release from, pH in relation to)

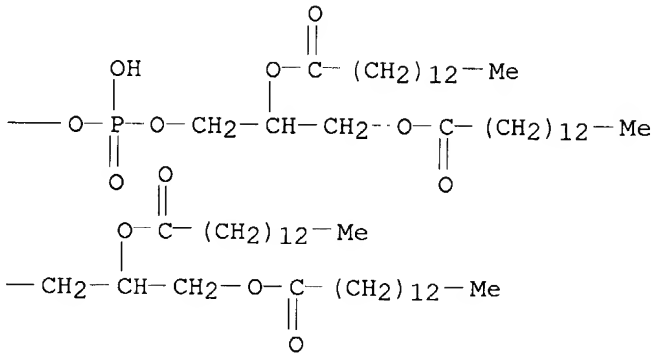
RN 97850-22-1 CAPLUS
CN 9,11,15-Trioxa-6-aza-10-phosphanonacosanoic acid, 4-[[4-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]amino]-10-hydroxy-5,16-dioxo-13-[(1-oxotetradecyl)oxy]-, 10-oxide (9CI) (CA INDEX NAME)

RN 97866-98-3 CAPLUS
CN Tetradecanoic acid, 11-[[4-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]amino]-5,19-dihydroxy-5,19-dioxido-10,14-dioxo-4,6,18,20-tetraoxa-9,15-diaza-5,19-diphosphatricosane-1,2,22,23-tetrayl ester (9CI) (CA INDEX NAME)

PAGE 1-A



PAGE 1-B



L8 ANSWER 7 OF 12 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1989:219079 CAPLUS
DOCUMENT NUMBER: 110:219079
TITLE: Aerosol containing liposomes and liposome-drug combinations
INVENTOR(S): Knight, Jack Vernon; Gilbert, Brian E.; Wilson, Samuel Z.; Six, Howard R.; Wyde, Philip R.
PATENT ASSIGNEE(S): Clayton Foundation for Research, USA
SOURCE: Eur. Pat. Appl., 11 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|

| | | | | |
|---|---|---|---|---|
| EP 267050 | A2 | 19880511 | EP 1987-309854 | 19871106 |
| EP 267050 | A3 | 19881026 | | |
| EP 267050 | B1 | 19940914 | | |
| R: AT, BE, CH, DE, ES, FR, GB, GR, IT, LI, LU, NL, SE | | | | |
| IL 84360 | A1 | 19920115 | IL 1987-84360 | 19871103 |
| NO 8704600 | A | 19880509 | NO 1987-4600 | 19871104 |
| CA 1263310 | A1 | 19891128 | CA 1987-551017 | 19871104 |
| DK 8705824 | A | 19880507 | DK 1987-5824 | 19871105 |
| FI 8704895 | A | 19880507 | FI 1987-4895 | 19871105 |
| AU 8780819 | A1 | 19880602 | AU 1987-80819 | 19871105 |
| AU 600766 | B2 | 19900823 | | |
| JP 63211223 | A2 | 19880902 | JP 1987-280853 | 19871106 |
| JP 2933931 | B2 | 19990816 | | |
| ES 2058124 | T3 | 19941101 | ES 1987-309854 | 19871106 |
| JP 11222423 | A2 | 19990817 | JP 1998-336266 | 19871106 |
| JP 3202704 | B2 | 20010827 | | |
| JP 11222424 | A2 | 19990817 | JP 1998-336267 | 19871106 |
| JP 3202705 | B2 | 20010827 | | |

PRIORITY APPLN. INFO.: US 1986-927898 A 19861106
JP 1987-280853 A3 19871106

AB Liposomes or liposome-drug combinations of heterogeneous size are reduced to a substantially homogeneous size by using an aerosol nebulizer; the liposome particles thus obtained have a diameter of <5 μ. Phosphatidylcholine 450 mg, chloroform 30 mL, and enviroxime 120 mg were mixed and the solvent was removed under vacuum and the lipid-drug mixture was dissolved in 60 mL of tert-BuOH. The solution was freeze-dried. Liposomes were prepared by resuspending the lyophilizate in 30 mL of H2O. The resulting liposomes were heterogeneous in size and had a diameter of ≤1-10 μm, they were passed through a nebulizer to reduce the size to ≤1 μ; the formulations may be supplied in com. available nebulizers. Enviroxime had no immune suppressive effect on the primary antibody response in mice, no cardiovascular effect on cats, and a depressive effect on the diastolic pressure in dogs.

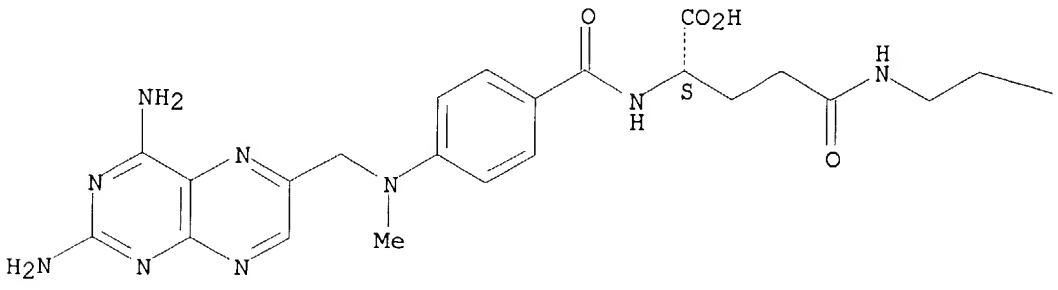
IT **97866-97-2 97866-98-3**
RL: BIOL (Biological study)
(aerosols containing, liposome-encapsulated)

RN 97866-97-2 CAPLUS

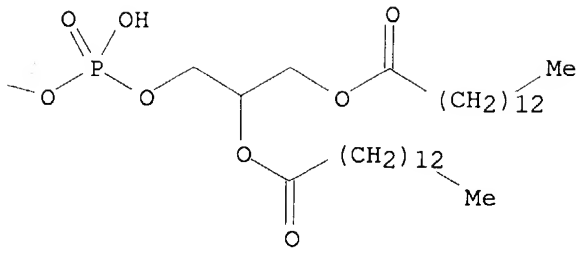
CN L-Glutamine, N2-[4-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-N-[4-hydroxy-4-oxido-10-oxo-7-[(1-oxotetradecyl)oxy]-3,5,9-trioxa-4-phosphatricos-1-yl]- (9CI) (CA INDEX NAME)

Absolute stereochemistry.

PAGE 1-A

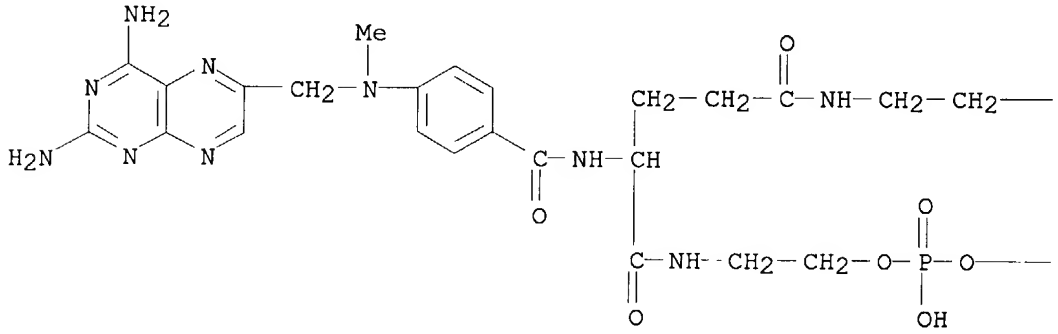


PAGE 1-B

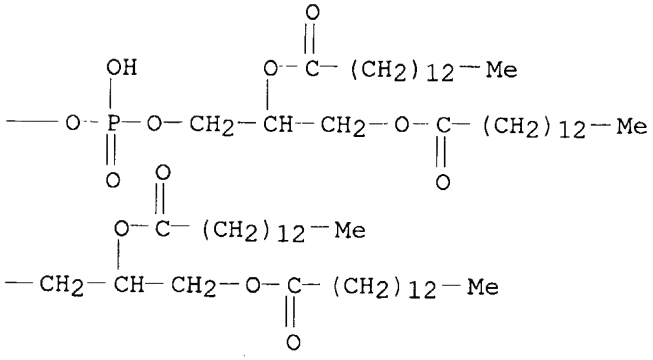


RN 97866-98-3 CAPLUS
CN Tetradecanoic acid, 11-[[4-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]amino]-5,19-dihydroxy-5,19-dioxido-10,14-dioxo-4,6,18,20-tetraoxa-9,15-diaza-5,19-diphosphatricosane-1,2,22,23-tetrayl ester (9CI) (CA INDEX NAME)

PAGE 1-A



PAGE 1-B



L8 ANSWER 8 OF 12 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1987:611449 CAPLUS
DOCUMENT NUMBER: 107:211449
TITLE: Circumvention of the methotrexate transport system by methotrexate-phosphatidylethanolamine derivatives:

effect of fatty acid chain length

AUTHOR(S): Kinsky, Stephen C.; Loader, Joan E.
CORPORATE SOURCE: Dep. Pediatr., Natl. Jew. Cent. Immunol. Respir. Med., Denver, CO, 80206, USA

SOURCE: Biochimica et Biophysica Acta (1987), 921(1), 96-103
CODEN: BBACAQ; ISSN: 0006-3002
DOCUMENT TYPE: Journal
LANGUAGE: English

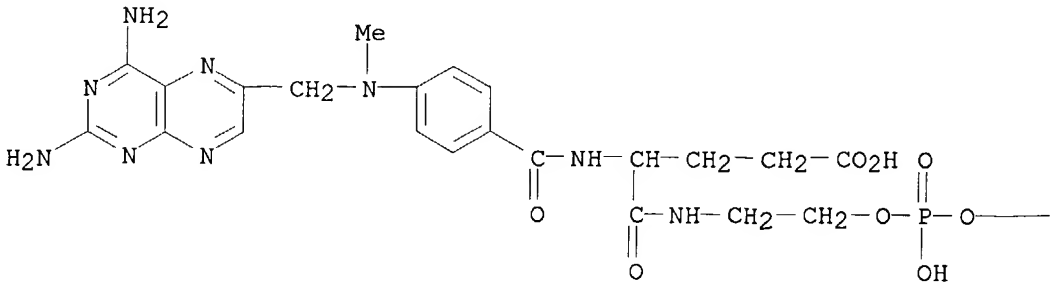
AB Methotrexate was conjugated via either the α- or γ-, or both α- and γ-glutamylcarboxyl groups to the amino function of dihexanoylphosphatidylethanolamine (C6C6PE) and 1-tetradecanoyl-2-hexanoylphosphatidylethanolamine (C14C6PE). These phospholipid prodrugs (either free or incorporated into liposomes) were compared with the corresponding ditetradecanoylphosphatidylethanolamine (C14C14PE) conjugates, some of whose properties have been described previously, for their ability to inhibit the proliferation of human leukemic cells (CEM/O) or cells derived therefrom (CEM/MTX) that are resistant to methotrexate because of a defective drug transport system. Regardless of chain length, the γ conjugates were more effective than either the α or the α,γ conjugates in inhibiting growth of the parent cells, confirming initial expts. with mouse cells. Chain length had, however, a pronounced influence on the capacity of the various γ derivs. to circumvent the transport defect. For example, CEM/MTX cells were 120-fold less susceptible than CEM/O cells to inhibition by either methotrexate or methotrexate-γ-C6C6PE, whereas both cell lines were equally sensitive to methotrexate-γ-C14C14PE. Although less potent than either of the foregoing, methotrexate-γ-C14C6PE could partially bypass the defective transport system. Methotrexate-γ-PE derivs. with appropriate acyl residues might be useful probes to investigate the mechanism by which phospholipids in general are able to traverse cell membranes.

IT **97850-22-1**, Methotrexate α-(ditetradecanoylphosphatidylethanolamine) **97866-97-2**, Methotrexate γ-(ditetradecanoylphosphatidylethanolamine) **97866-98-3**, Methotrexate α,γ-(ditetradecanoylphosphatidylethanolamine) **111318-45-7** **111318-46-8** **111318-47-9**, Methotrexate γ-(1-tetradecanoyl-2-hexanoylphosphatidylethanolamine) **111318-48-0**, Methotrexate α,γ-(1-tetradecanoyl-2-hexanoylphosphatidylethanolamine) **111348-68-6**, Methotrexate γ-dihexanoylphosphatidylethanolamine **111348-69-7**, Methotrexate α,γ-dihexanoylphosphatidylethanolamine
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(neoplasm inhibition by, drug transport in relation to, in humans)

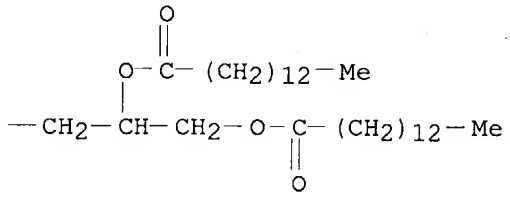
RN 97850-22-1 CAPLUS

CN 9,11,15-Trioxa-6-aza-10-phosphanonacosanoic acid, 4-[[4-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]amino]-10-hydroxy-5,16-dioxo-13-[(1-oxotetradecyl)oxy]-, 10-oxide (9CI) (CA INDEX NAME)

PAGE 1-A



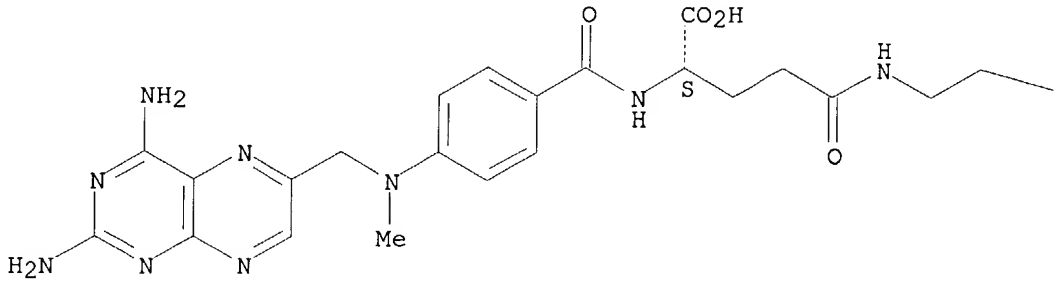
PAGE 1-B



RN 97866-97-2 CAPLUS
CN L-Glutamine, N2-[4-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-N-[4-hydroxy-4-oxido-10-oxo-7-[(1-oxotetradecyl)oxy]-3,5,9-trioxa-4-phosphatricos-1-yl]- (9CI) (CA INDEX NAME)

Absolute stereochemistry.

PAGE 1-A



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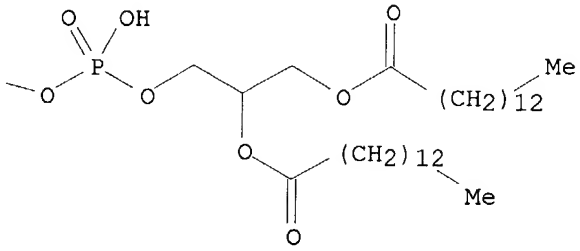

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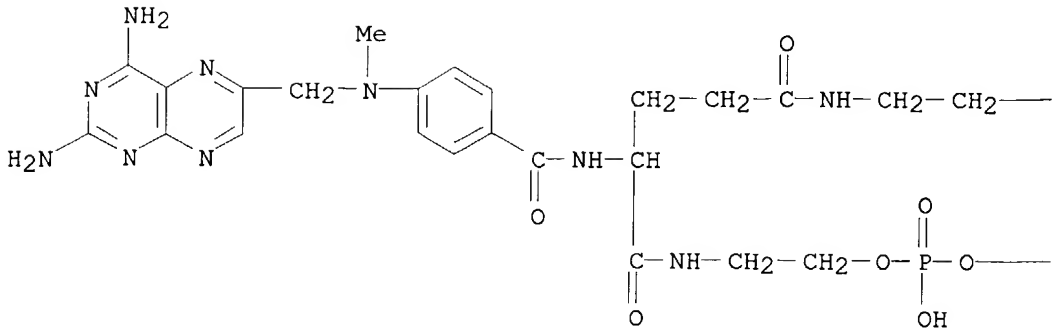
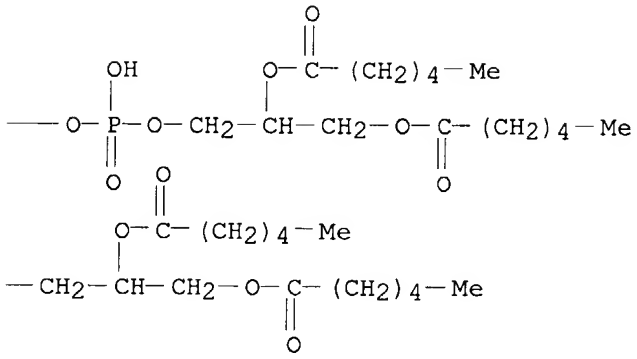


PAGE 1-B



L8 ANSWER 9 OF 12 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1987:149056 CAPLUS
DOCUMENT NUMBER: 106:149056
TITLE: Inhibition of cell proliferation by putative metabolites and non-degradable analogs of methotrexate-γ-dimyristoylphosphatidylethanolamine
AUTHOR(S): Kinsky, Stephen C.; Loader, Joan E.; Hashimoto, Keiichiro
CORPORATE SOURCE: Dep. Pediatr., Natl. Jew. Cent. Immunol. Respir. Med., Denver, CO, 80206, USA
SOURCE: Biochimica et Biophysica Acta (1987), 917(2), 211-18
CODEN: BBACAQ; ISSN: 0006-3002
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Previous investigations have shown that untargeted liposomes, in which methotrexate is anchored to the lipid bilayers as methoxtrexate-γ-dimyristoylphosphatidylethanolamine (methotrexate-γ-DMPE), can inhibit in vitro cell proliferation. To test the possibility that this inhibition may involve extracellular metabolism of methotrexate-γ-DMPE, it was degraded chemical (dilute alkali) or enzymically (phospholipase A2, phospholipase C, phospholipase C plus phosphatase), and the products were assayed using human lymphoblastoid T cells or a subline that has a defective methotrexate transport system. Neither methotrexate-γ-(1-

myristoyl)-glycerophosphorylethanolamine [107646-99-1], methotrexate-γ-glycerophosphorylethanolamine [97850-20-9], methotrexate-γ-phosphorylethanolamine [107647-00-7], nor methotrexate-γ-ethanolamine [81919-36-0] resemble methotrexate-γ-DMPE sensitized liposomes or the free derivative in their ability to block tritiated deoxyuridine incorporation into DNA. When added extracellularly, these putative metabolites manifest a higher ID50 concentration and/or, unlike the liposomes or unincorporated methotrexate-γ-DMPE, utilize the methotrexate transport system to enter cells. Addnl., the authors synthesized methotrexate-γ-dihexadecylphosphatidylethano lamine [107646-97-9] and methotrexate-γ-hexadecylphosphorylethanolamine [107646-98-0], analoges of methotrexate-γ-DMPE that cannot be hydrolyzed by phospholipases A2, C and D; liposomes prepared with these derivs. are markedly less potent cytotoxic agents than methotrexate γ-DMPE sensitized liposomes. Apparently, methotrexate-γ-DMPE must undergo intracellular metabolism to exert optimal inhibition; on possible mechanisms by which methotrexate-γ-DMPE may enter cells are also indicated.

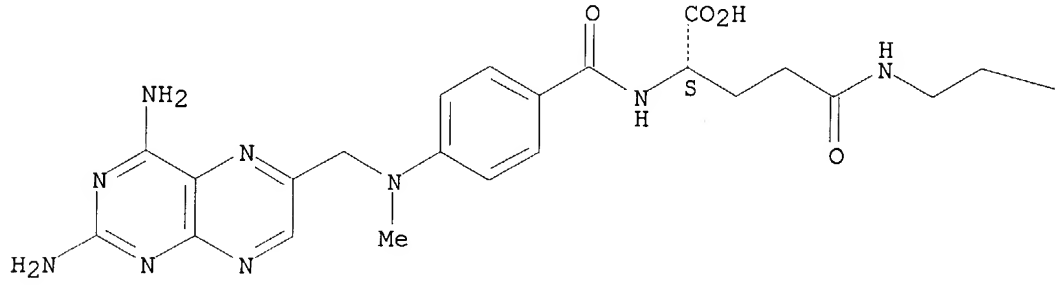
IT **97866-97-2D**, analogs and metabolites
RL: ADV (Adverse effect, including toxicity); BIOL (Biological study)
(cytotoxicity of)

RN 97866-97-2 CAPLUS

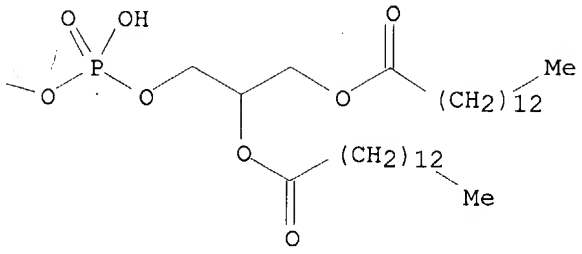
CN L-Glutamine, N2-[4-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-N-[4-hydroxy-4-oxido-10-oxo-7-[(1-oxotetradecyl)oxy]-3,5,9-trioxa-4-phosphatricos-1-yl]- (9CI) (CA INDEX NAME)

Absolute stereochemistry.

PAGE 1-A



PAGE 1-B



L8 ANSWER 10 OF 12 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1986:161633 CAPLUS
DOCUMENT NUMBER: 104:161633
TITLE: Effect of liposomes sensitized with methotrexate-γ-dimyristoylphosphatidylethanolami

ne on cells that are resistant to methotrexate

AUTHOR(S): Kinsky, Stephen C.; Hashimoto, Keiichiro; Loader, Joan E.; Knight, Marcia S.; Fernandes, Daniel J.

CORPORATE SOURCE: Dep. Pediatr., Natl. Jew. Cent. Immunol. Respiratory Med., Denver, CO, 80206, USA

SOURCE: Biochimica et Biophysica Acta (1986), 885(2), 129-85
CODEN: BBACAQ; ISSN: 0006-3002

DOCUMENT TYPE: Journal

LANGUAGE: English

AB This study compares the ability of methotrexate (MTX) [59-05-2] and liposomes, in which the drug is anchored to the lipid bilayers via methotrexate-γ-dimyristoylphosphatidylethanolamine (MTX-γ-DMPE) [ **97866-97-2**], to inhibit proliferation of human leukemic cells (CEM/O) and cells derived from this line that are resistant to methotrexate because of either a defective transport system (CEM/MTX cells) or elevated levels of dihydrofolate reductase (CEM/R1 cells). Whereas CEM/O and CEM/MTX cells showed a 120-fold difference in their susceptibility to methotrexate (as measured by the incorporation of tritiated deoxyuridine into DNA), both lines were equally sensitive to the liposomes. In contrast, proliferation of CEM/MTX cells was not inhibited significantly by methotrexate-γ-glycerophosphorylethanolamine (MTX-γ-glyceroPE) [97850-20-9], the water-soluble analog of MTX-γ-DMPE. Both the ability of the liposomes to circumvent the transport defect, and the inability of MTX-γ-glyceroPE to do so, were anticipated on the basis of previous expts. which showed that thiamine pyrophosphate could antagonize inhibition of mouse 3T3 and L1210 cell proliferation by methotrexate and MTX-γ-glyceroPE, but not inhibition by liposomes. Human cells (CEM/O) behaved similarly. The present expts. also suggest that liposomes prepared with MTX-γ-DMPE can partially reverse the methotrexate resistance of CEM/R1 cells that is due to overprodn. of the target enzyme.

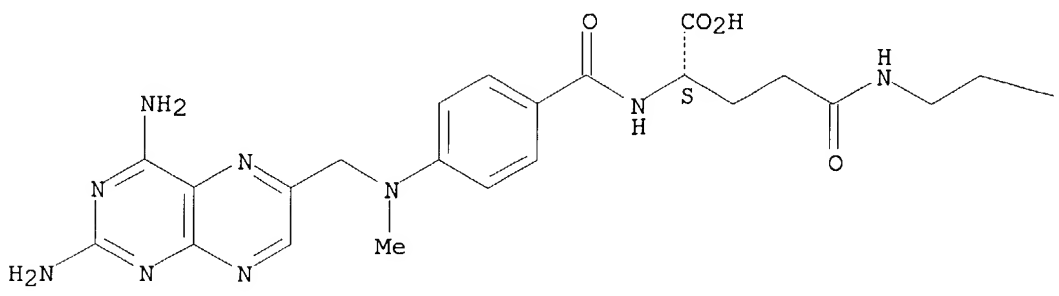
IT **97866-97-2**
RL: BIOL (Biological study)
(cytotoxicity of liposomes containing, to human leukemia cells resistant to methotrexate)

RN 97866-97-2 CAPLUS

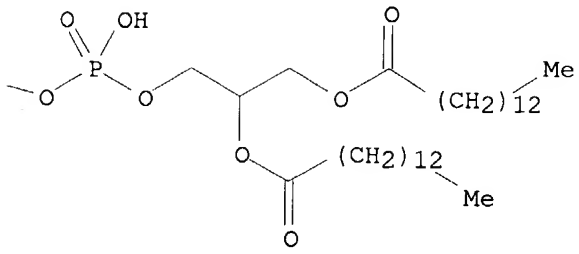
CN L-Glutamine, N2-[4-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-N-[4-hydroxy-4-oxido-10-oxo-7-[(1-oxotetradecyl)oxy]-3,5,9-trioxa-4-phosphatricos-1-yl]- (9CI) (CA INDEX NAME)

Absolute stereochemistry.

PAGE 1-A



PAGE 1-B



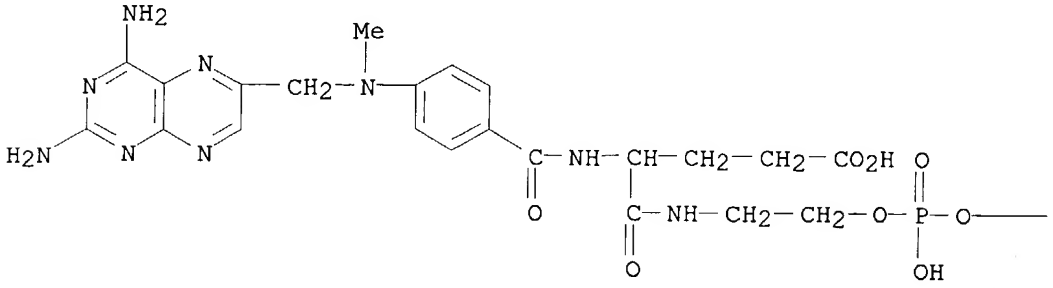
L8 ANSWER 11 OF 12 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1985:566010 CAPLUS
DOCUMENT NUMBER: 103:166010
TITLE: Synthesis and characterization of methotrexate-dimyristoylphosphatidylethanolamine derivatives and the glycerophosphorylethanolamine analogs
AUTHOR(S): Hashimoto, Keiichiro; Loader, Joan E.; Kinsky, Stephen C.
CORPORATE SOURCE: Dep. Pediatr., Natl. Jewish Hosp., Denver, CO, 80206, USA
SOURCE: Biochimica et Biophysica Acta (1985), 816(1), 163-8
CODEN: BBACAQ; ISSN: 0006-3002
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Three methotrexate (MTX) [59-05-2] derivs. of dimyristoylphosphatidylethanolamine (DMPE) [20255-95-2] were prepared by conjugation of the α and/or γ-glutamyl-carboxyl groups of the drug with the amino function of the phospholipid. These derivs. were characterized anal. and chromatog. as MTX-γ-DMPE [ **97866-97-2**], MTX-α-DMPE [ **97866-97-2**], and MTX-α,γ-diDMPE [ **97866-98-3**]. The corresponding glycerophosphorylethanolamine [1190-00-7] analogs were also prepared and identified. The biol. properties of these compds. are under investigation.

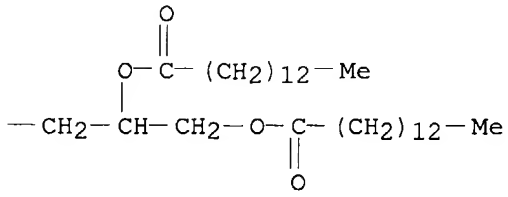
IT **97850-22-1P 97866-97-2P 97866-98-3P**
RL: PRP (Properties); SPN (Synthetic preparation); PREP (Preparation)
(preparation and properties of, for liposome delivery)

RN 97850-22-1 CAPLUS
CN 9,11,15-Trioxa-6-aza-10-phosphanonacosanoic acid, 4-[[4-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]amino]-10-hydroxy-5,16-dioxo-13-[(1-oxotetradecyl)oxy]-, 10-oxide (9CI) (CA INDEX NAME)

PAGE 1-A



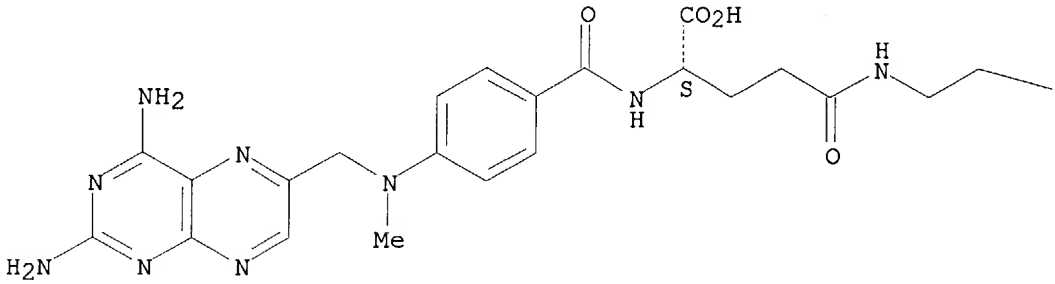
PAGE 1-B



RN 97866-97-2 CAPLUS
CN L-Glutamine, N2-[4-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-N-[4-hydroxy-4-oxido-10-oxo-7-[(1-oxotetradecyl)oxy]-3,5,9-trioxa-4-phosphatricos-1-yl]- (9CI) (CA INDEX NAME)

Absolute stereochemistry.

PAGE 1-A



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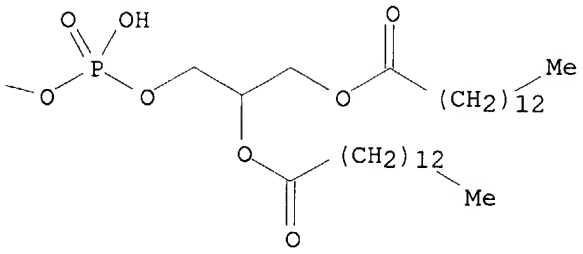
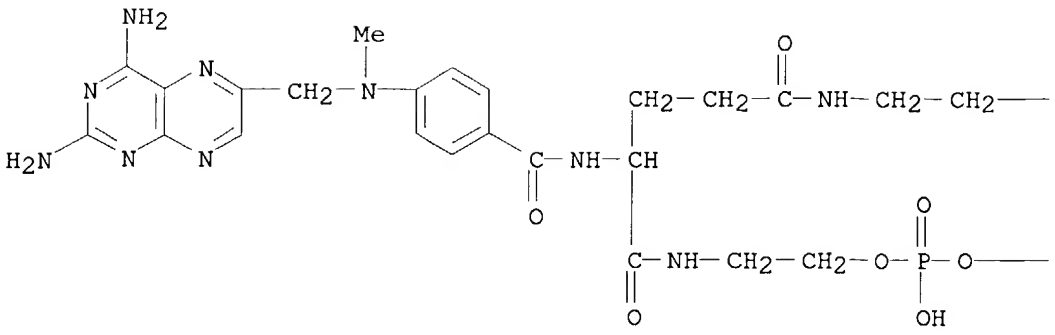
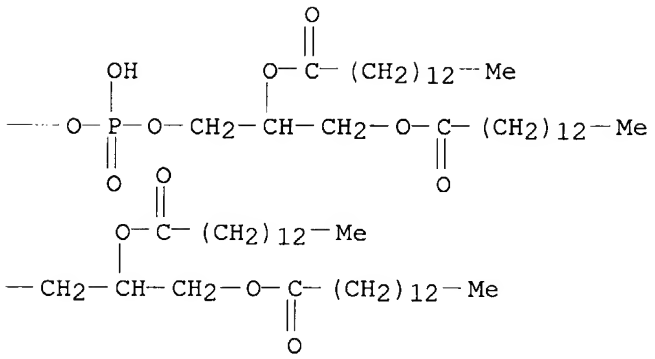


RN 97866-98-3 CAPLUS
CN Tetradecanoic acid, 11-[[4-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]amino]-5,19-dihydroxy-5,19-dioxido-10,14-dioxo-4,6,18,20-tetraoxa-9,15-diaza-5,19-diphosphatricosane-1,2,22,23-tetrayl ester (9CI) (CA INDEX NAME)

PAGE 1-A



PAGE 1-B



L8 ANSWER 12 OF 12 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1985:498390 CAPLUS
DOCUMENT NUMBER: 103:98390
TITLE: Inhibition of cell proliferation and dihydrofolate reductase by liposomes containing methotrexate-dimyristoylphosphatidylethanolamine derivatives and by the glycerophosphorylethanolamine analogs
AUTHOR(S): Hashimoto, Keiichiro; Loader, Joan E.; Knight, Marcia S.; Kinsky, Stephen C.
CORPORATE SOURCE: Dep. Pediatr., Natl. Jewish Hosp., Denver, CO, 80206, USA
SOURCE: Biochimica et Biophysica Acta (1985), 816(1), 169-78
CODEN: BBACAQ; ISSN: 0006-3002
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Liposomes, which were prepared with the three methotrexate (MTX)-dimyristoylphosphatidylethanolamine (DMPE) derivs. were tested for their ability to block proliferation of mouse 3T3 and L1210 cells. Tritiated deoxyuridine incorporation into DNA could be completely inhibited by liposomes sensitized with MTX-DMPE I (MTX-γ-DMPE) [ **97866-97-2**]. Under similar conditions, liposomes containing MTX-DMPE II (MTX-α-DMPE) [ **97850-22-1**] and MTX-DMPE III (MTX-α,γ-diDMPE) [ **97866-98-3**] produced partial and no inhibition, resp. These effects on cell growth were paralleled by the

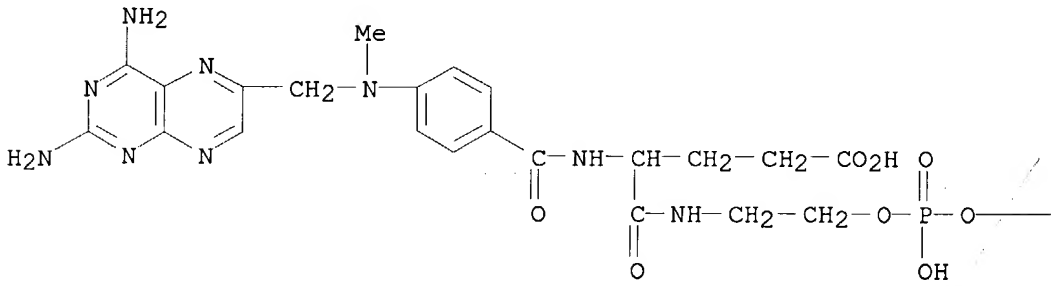
capacity of liposomes, prepared with each of the DMPE derivs., to inhibit dihydrofolate reductase [9002-03-3] isolated from L1210 cells. Analogous expts. with the three corresponding glycerophosphorylethanolamine (glyceroPE) analogs also indicated that MTX-glyceroPE I was the most effective inhibitor of both cell proliferation and enzymic activity. However, MTX-DMPE I sensitized liposomes apparently enter target cells as a consequence of phagocytosis, and not via the ubiquitous methotrexate transport system that is employed by MTX-glyceroPE I. For example, novel use of histamine pyrophosphate [154-87-0] showed that this compound had no influence on inhibition of cell proliferation due to liposomes, whereas thiamine pyrophosphate could completely antagonize the inhibitory effects of methotrexate and MTX-glyceroPE I. The results are discussed with reference to possible therapeutic advantages of these liposomes.

IT **97850-22-1 97866-97-2 97866-98-3**
RL: BIOL (Biological study)
(cytotoxicity of and dihydrofolate reductase inhibition by)

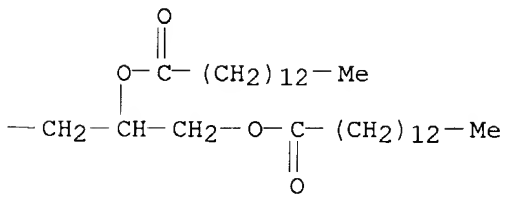
RN 97850-22-1 CAPLUS

CN 9,11,15-Trioxa-6-aza-10-phosphanonacosanoic acid, 4-[[4-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]amino]-10-hydroxy-5,16-dioxo-13-[(1-oxotetradecyl)oxy]-, 10-oxide (9CI) (CA INDEX NAME)

PAGE 1-A



PAGE 1-B



RN 97866-97-2 CAPLUS

CN L-Glutamine, N2-[4-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-N-[4-hydroxy-4-oxido-10-oxo-7-[(1-oxotetradecyl)oxy]-3,5,9-trioxa-4-phosphatricos-1-yl]- (9CI) (CA INDEX NAME)

Absolute stereochemistry.